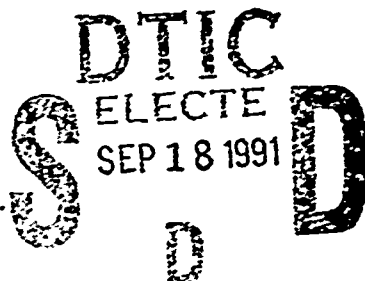**AD-A240 465**

HYDROCARBON DEGRADATION POTENTIAL IN REFERENCE SOILS

AND SOILS CONTAMINATED WITH JET FUEL

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INTRODUCTION

A series of test wells were drilled adjacent to a fuel farm and a JP-5 jet fuel pump station located at a naval air station in Maryland. At least 5 hectares of subsurface soil (to an average depth of 4 m) above a local aquifer were found to contain high concentrations of petroleum compounds, including such volatile aromatics as benzene, toluene, ethylbenzene, and xylenes. Horizontal transport has resulted in slow seepage from banks into streams of the affected area. The source of the petroleum is due to various spills over the past 10 years and possibly continuous leakage from the tanks.

There is a large body of literature describing the microbial metabolism of polycyclic aromatic hydrocarbons in aerobic solid-water systems (Arvin et al. 1988; Atlas 1981; Bauer and Capone 1985; Mihelcic and Luthy 1988; Swindoll et al. 1988; Van der Hoek et al. 1989). Petroleum degradation in surface and subsurface soils is affected by such factors as moisture content, pH, soil type, soil organics, temperature, and oxygen concentrations. We determined the degradation rates of ^{14}C-labeled hydrocarbons added to soils collected from a contaminated surface site (Site D), contaminated subsurface sites (Wells A and B), and a clean reference site (Well C). The radiolabeled hydrocarbons used include benzene, toluene, naphthalene, 1-methynaphthalene, phenanthrene, fluorene, anthracene, chrysene, and hexadecane.

Microbial degradation rates were based on determination of mineralization rates (production of $^{14}CO_2$) of hydrocarbons that were added to soil samples. This technique, often referred to as hydrocarbon degradation potential, has been used to evaluate hydrocarbon degradation in natural waters and soils (Atlas 1979; Lee and Ryan 1983; Scheunert et al. 1987). Since water was added and oxygen was not limiting, the hydrocarbon rates determined

are likely to be higher than those occurring *in situ*. Using radiolabeled hydrocarbons, information can be provided on differences in the degradation rates of various petroleum compounds in different types of soils at a site, on possible production of petroleum metabolites in the soil, and on the importance of anaerobic petroleum degradation and the effects of nutrient, water, and surfactant addition on biodegradation rates.

MATERIALS AND METHODS

Test wells (A, B and C) were augured to a clay confining layer. We used core samples from above the observed water table. A few samples from the cores were collected by aseptic techniques (i.e., undisturbed, sealed samples) to compare with soils not aseptically collected. Gas samples were withdrawn from a depth of 1 m from the top of the casing prior to water sample retrieval. Volatile hydrocarbons were determined using a field photoionization detection-gas chromatograph. Well waters were also analyzed using a gas chromatograph/mass spectrometer. These analyses were conducted by International Technology Corporation under contract to the Naval Civil Engineering Laboratory (Port Hueneme, California). Standards used included benzene, toluene, xylenes, and ethylbenzene. ^{14}C-labeled hydrocarbons were added to soil mixed with water (5 g soil with 10 mL water) in 250 mL flasks capped with silicon stoppers. After incubation at room temperature (20 C the respired ^{14}CO$_2$ was collected by trapping on phenethylamine paper and counted in a liquid scintillation counter (for details of this procedure see Lee and Ryan 1983). Controls were soil samples containing 10 percent formalin. All samples were in triplicate for each time interval

Degradation can often be expressed by the first-order equation

$$dc/dt = kc \qquad (1)$$

where k is the rate constant and c is the concentration of the hydrocarbon at time t. Half-lives were calculated by the equation

$$t_{1/2} = \frac{0.693}{k} \qquad (2)$$

Radiolabeled hydrocarbon used included 2-[8-^{14}C] methylnaphthalene (136.9 MBq/mM); 1-[^{14}C]-naphthalene (135.8 MBq/mM); 9-^{14}C-fluorene (95.1 MBq/mM); ^{14}C-methylbenzene (647 MBq/mM); 5,6 (11,12-^{14}C) chrysene (2.33.1 MBq/mM); 9-anthracene (76.6 MBq/mM); UL-^{14}C-benzene (370 MBq/mM); and ^{14}C-hexadecane (2268 MBq/mM).

RESULTS

Soils at the various sites are primarily sand, but Site D has surface seeps and includes a layer of oil-saturated peat. The concentrations of somce selected volatile hydrocarbons at the study sites is given in Table 1. Soils from Wells A and B were contaminated with benzene but no toluene was detected. These wells are adjacent to a previously leaking tank and JP-5 and JP-5 usually has low toluene concentrations. Site D and a nearby seep are adjacent to a fuel farm and tanks here have held JP-4, JP-5, fuel oil #2, and AVGAS. Fuel oil, JP-4, and AVGAS all contain toluene, and benzene and thus both toluene and benzene were present in the seep soils. Site D, where the soil was collected near the surface, appears to be contaminated by an old spill since benzene was absent and toluene concentration was very low and mostly high molecular weight alkanes were found. Soil taken from cores at depths of 2 to 3 m from Wells A, B, and C had petroleum hydrocarbon concentrations of 570, 530, and less than 20 µg/g soil, respectively.

Soil from Wells A and B rapidly degraded benzene. toluene, naphthalene, 1-methylnaphthalene, and phenanthrene with half-lives ranging from 1 to 3 days (Figs 1-4)

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Degradation of these compounds in "clean" soil from reference Well C was very low with half-lives for these compounds ranging from 20 to 125 days (Fig. 1-4). No differences in degradation potential were found between soils collected by aseptic or non-aseptic techniques. At Site D, hydrocarbon degradation rates were lower in oiled peat compared with oiled sand with naphthalene half-lives ranging from 0.7 to 2.5 days, (Figs. 1, 2, and 3). Toluene was more slowly degraded in soil from Well B than in soil from Site D (Fig. 1). This may be due to the absence of toluene from soil at Well B so that the microflora at this site was not adapted to toluene, even though other aromatics were rapidly degraded including benzene, naphthalene, and methylnaphthalene (Fig. 2 and 3). Fluorene, chrysene, and anthracene were only slowly degraded in both oiled soils and reference soils (data not shown). These compounds are in low concentrations in JP-4, JP-5, and AVGAS. Hexadecane, a long chain alkane which can be produced by plants and is normally found in soil, was degraded at a much higher rate in reference soil compared with oiled soils. This hydrocarbon is in low concentrations in jet fuels. In addition to the type of soil (i.e., peat or sand), the amount of water in the soil was shown to be important. Soil with a moisture content of 20 percent degraded naphthalene at a lower rate than contaminated sand with a moisture content of 66 percent (Fig. 6).

DISCUSSION

Soils contaminated with petroleum are characterized by high concentrations of hydrocarbon-degrading bacteria (Dragum 1988, Raymond et al. 1976). Oil was added to one study site and after 8 months, the soil showed a 10-fold increase in the concentration of oil-degrading bacterial (Pinholt et al 1979). Thus, soils previously exposed to various foreign compounds, including pesticides or petroleum, show an enhanced ability to degrade such

compounds or mixtures (Aurelius and Brown 1987; Chapman *et al.* 1986; Harris *et al.* 1988; Heitkamp *et al.* 1987; Hendry and Richardson 1988; Lee and Ryan 1983; Lee *et al.* 1988). The lag period found in "clean" reference soils before hydrocarbon degradation begins is assumed to be due to the time needed for the microbial community to adapt to the added hydrocarbons (Cripe *et al.* 1987). Such adaptation has been defined as a change in the microbial community that increases the rate of transformation of a compound as a result of prior exposure to the compound (Spain and Van Veld 1983). The time needed for this adaptation depends on the compounds and can vary from days to weeks. Thus, because of such adaptation at our study site, we observed rapid degradation of toluene, benzene, naphthalene, methylnaphthalene, and phenanthrene.

Work with pure cultures has shown that oil degraders only slowly degrade certain xylene (dimethybenzene) isomers and highly branched alkanes because of steric hindrances inhibiting the ability of the bacterial oxygenases to attack the compounds (Bailey *et al.* 1973; Hopper 1978). The p- and m-xylenes were readily degraded by pure cultures but o-xylene was only slowly degraded. Therefore, even after extensive degradation of petroleum, certain highly branched aromatics and alkanes can remain in the soil. Analysis of the petroleum-contaminated soils at the study sites indicated that the major compounds present were branched-chain alkanes (Hoeppel 1988). The degradation we report here are potential rates and are probably not the rates occurring in the subsurface soils, but indicate a degradation rate that can be obtained when such factors as moisture and oxygen are optimal Naphthalene added to sediments from a stream heavily contaminated with petroleum had half-lives of less than 1 day (Herbes and Schwall 1978), while addition to less contaminated sediments resulted in a 17 day half-life (Heitkamp *et al.* 1987).

An interesting observation was the very slow degradation of certain hydrocarbons that were present in very low or nondetectable levels in the contaminated soils. It appeared that the microbes were adapted to degrade only the compounds in the petroleum products at the site. Thus, toluene was only slowly degraded in a site contaminated with JP-5 (very low in toluene content), while benzene, naphthalene, and methylnaphthalene were rapidly degraded in soil from this site.

The data indicated that under the conditions used in our incubations, microflora of the contaminated sites had the potential to rapidly degrade many of the most toxic components found in jet fuels contaminating the area. From a practical standpoint, it appears that it is unnecessary to add hydrocarbon degrading microbes to such contaminated sites, but rather a need to optimize biological degratation by monitoring and modifying oxygen, nutrients, and moisture. (For summaries of work on stimulating biodegradion of petroleum in soils, see Lee *et al.* 1988; Morgan and Watkinson, 1990.)

ACKNOWLEDGEMENTS

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TABLE 1

Volatile Hydrocarbons Found in Soil Cores
or Surface Soils at
Naval Air Station on Patuxent River, Maryland[1]

Compounds (μg/L)	Site D	Well B (μg/L)	Well A	Well C	Seep[2]
Volatile Alkanes	----	2535	2487	2	11,000
Benzene	N.D.[3]	850	1020	N.D.	1,400
Ethylbenzene	26	430	23	2	7,000
Toluene	6	N.D.	N.D.	N.D.	5,600
m, p, o-xylenes	186	240	1260	N.D.	N.D.

[1]Site D and nearby seep are adjacent to fuel farm containing JP-4, JP-5, fuel oil #2, and AVGAS; Wells A and B are adjacent to previously leaking JP-5 tank; Well C is from a reference site where there was no evidence of contamination from the fuel tanks.

[2]μg/g soil

[3]Not Detected <1 μg/L

FIGURE LEGENDS

Fig. 1 ^{14}C-toluene added to soil (5 μg/5 g soil) from Site D and Wells B and C. Error bars are standard deviation (n=3). Calculated half-lives for toluene in Well B and C were 7 and 50 days, respectively. Calculated half-lives for toluene in sand and peat of Site D were 0.6 and 1.9 days, respectively.

Fig. 2 ^{14}C-benzene added to soil (5 μg/5 g soil) from Site D and Wells B and C. Error bars are standard deviation (n=3). The calculated half-lives of benzene in Wells B and C were 5 and 125 days, respectively. The calculated half-lives of benzene in sand and peat of Site D were 6 and 41 days, respectively.

Fig. 3 ^{14}C-naphthalene and ^{14}C-methyinaphthalene added to subsurface soils (5 μg/5 g soil) from Wells B and C. Error bars are standard deviation (n=3). Calculated half-lives for naphthalene in Wells B and C were 0.7 and 20 days, respectively. Calculated half-lives for methylnaphthalene in Wells B and C were 1.2 and 25 days, respectively.

Fig. 4 ^{14}C-phenanthrene added to soil (5 μg/5 g soil) from Site D and Wells B and C. Error bars are standard deviation (n=3). Calculated half-lives for phenanthrene in soils of Site D, Wells B and C were 10, 6 and 21 days, respectively.

Fig. 5   Effect of moisture content on ^{14}C-naphthalene degradation. ^{14}C-naphthalene added to subsurface soil (5 μg/5 g soil) from Well B (oiled). Error bars are standard deviation (n=3).

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Figure legend:

△ site D. sandy (oil contaminated)

□ site D. peat

o well B. sandy (oil contaminated)

● well C. sandy (reference soil)

Y-axis: $^{14}CO_2$ formed (% of total)

X-axis: Time (hrs)

- △ subsurface oiled sand well B
- □ oiled sand. site D
- ○ oiled peat. site D
- ● reference subsurface sand. well C

- ◡ ¹⁴C-naphthalene well B (oiled sand)
- ☐ ¹⁴C-methylnaphthalene well B
- △ ¹⁴C-naphthalene well C (refe ence soil)
- ● ¹⁴C-methylnaphthalene well C

$^{14}CO_2$ formed (% of total)

Time (hrs)

Figure showing $^{14}CO_2$ formed (% of total) versus Time (hrs).

Legend:
△ ^{14}C-naphthalene in 5g soil plus 10ml of water
○ ^{14}C-naphthalene in 5g soil plus 2ml of water